SCIENTIFIC PAPER

Chitosan Hemostatic Dressing for Renal Parenchymal Wound Sealing in a Porcine Model: Implications for Laparoscopic Partial Nephrectomy Technique

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ABSTRACT

Background and Objectives: This study was to evaluate the feasibility of using a novel chitosan hemostatic dressing to control hemorrhage and urinary leakage by sealing off the parenchymal wound following LPN.

Methods: Nine heparinized domestic swine underwent bilateral laparoscopic partial nephrectomies involving either a polar or wedge resection. Estimated blood loss (EBL), hemostatic score, operative time, and adhesion score of the chitosan dressing were documented during LPN. Retrograde pyelography was performed to assess urinary leakage.

Results: Of 18 procedures, complete hemostasis after deployment of the chitosan dressing was successfully achieved in 17 of them. The hemostasis score improved significantly after the deployment in both polar ($P<0.001$) and wedge ($P=0.017$) resections. The rate of successful pyelocaliceal sealing was 85% (11/13) in polar and 60% (3/5) in wedge resections. Application of a bandage in wedge resections was fraught with greater difficulties in terms of number of applications required and prolonged operative time. However, the differences between this group and polar resection were not statistically significant.

Conclusions: The chitosan hemostatic dressing is capable of being used in LPN procedures as a primary or supplemental material for controlling parenchymal hemorrhage and sealing the renal collecting system in the animal model.

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Key Words: Chitosan, Hemostasis, Nephrectomy, Laparoscopic.

INTRODUCTION

Laparoscopic partial nephrectomy (LPN) has become a standard procedure in patients with small renal tumors, but the procedure remains technically challenging largely due to the lack of a reliable method for obtaining consistent parenchymal hemostasis and the difficulties in obtaining secure suture closure of the renal collecting system.[1] Urinary leakage and hemorrhage-related complication rates of LPN remain significantly higher than that of open partial nephrectomy.[2,3] At the present time, various topical sealants including fibrin glue,[4] gelatin resorcinol formaldehyde glue,[5] and oxidized cellulose or gelfoam sponges[1] are frequently used alone or in conjunction with specialized instrumentation and agents to acquire hemostasis. Although each of these techniques has merit, none of them has been proven to be the ideal method for performing LPN.[6] Recently, a novel chitosan-based hemostatic dressing has been shown to efficiently control aggressive hemorrhaging from severe traumatic injuries in animal models.[7] In this study, we evaluated whether the chitosan-based hemostatic dressing can provide a watertight wound seal to control renal parenchymal bleeding and urine leakage during LPN.

METHODS

The chitosan-based hemostatic dressings (HemCon Medical Technologies Inc, Portland, OR) were prepared by freeze-drying dilute aqueous acetic acid solutions of ultrapure grades of chitosan (FMC NovaMatrix, Iceland) in Teflon-coated aluminum molds. The resultant sponges were compressed, annealed, and gamma-irradiated to yield sterile, dissolution resistant, and adhesive dressings. These sponges were 58 mm in diameter and between 1.5-mm to 1.85-mm thick with a final chitosan density between 0.12 g/cm^3 and 0.15 g/cm^3. Prior to irradiation, a 50-μ m compliant bioabsorbable film (TephaFLEX polymer, Tepha Inc., Lexington, MA) was adhered to one side of the sponge as a waterproof backing to act as reinforce-

ment and to reduce adhesiveness of the sponge to surgical instruments. The backing side faces up on application, and the other side, or "active side," is the side directly applied to the wound surface.

Nine crossbred adult domestic swine, both sexes, average body weigh 56kg (range, 50 to 76) were included in this study. All procedures for handling and caring for the animals were carried out in accordance with the 1996 National Research Council's "Guide for the Care and Use of Laboratory Animals," and approved by the Institutional Animal Care and Use Committee of our research institution.

After induction of general anesthesia, the animal was placed in a lateral position, and CO_2 pneumoperitoneum was created using a 14-gauge Veress needle. Four working ports were placed as follows[1]: a 5-mm port at the midclavicular line 3 inches above the umbilicus[2]; a 5-mm 1 inch below the umbilicus in the line joining to the anterior superior iliac spine[3]; a 10-mm port 2 inches horizontal above the umbilicus for a 45° laparoscope; and[4] a 10-mm port midway between the first 5-mm port and the third 10-mm port for the introduction of the chitosan dressing and utilization of a 10-mm fan retractor. A bolus of 5000 units of intravenous heparin was given 10 minutes before the operation, and additional bolus dosages of 1000 units were given intraoperatively every 30 minutes as required to maintain the activated clotting time (ACT) over 200 seconds and rechecked throughout the surgical procedure. ACT was rechecked every 30 minutes. If ACT dropped below 200, an additional 1000 units of heparin was regiven until it was above 200.

The kidney was identified, and following exposure of an upper pole (N=5), a lower pole (N=8), or a wedge (N=5), resection was performed using a Harmonic scalpel (Ethicon Endosurgery, Cincinnati, OH) without hilar occlusion. In the polar resection, at least one third of the renal tissue was resected; in the wedge resection, tissue approximately 3 cm in depth and 3 cm in width was removed from the middle of the kidney, making sure by visual confirmation that the collecting system was entered. The hemorrhage through the parenchymal surface of the kidney was assessed visually by assigning a 0–4 hemostatic score as described previously (0=no hemostasis; 1=steady bleeding; 2=moderate bleeding; 3=mild oozing; and 4=dry).[8] A chitosan dressing (6-cm diameter round shape) was furled and delivered through a 10-mm port, unfurled, and deployed onto the renal resected surface with gentle compression using a 10-mm fan retractor (US Surgical, Norwalk, CT) for 3 minutes **(Figure 1)**. The hemostatic score was recorded again. In case of incomplete hemostasis (hemostatic score ≥2), the chitosan dressing was removed, and a second piece was deployed as described above. The number of attempts required for successful deployment was recorded. After completion of satisfactory hemostasis and secure adhesion of the dressing on one side, the animal was turned onto the contralateral side and the procedure was repeated.

Once both sides had achieved initial hemostasis, the abdomen was deflated and an additional 30 minutes were used to observe hemostasis stability. Pneumoperitoneum was then reestablished, and the repair sites were reassessed laparoscopically for any evidence of rebleeding or urinary extravasation and to obtain the final hemostatic score. A retrograde pyelography was performed with bilateral ureter catheterization via cystotomy to assess the integrity of the collecting system and pyelocaliceal urinary leakage. Finally, the animals were euthanized and both the kidneys were removed through midline laparotomy for gross assessment of the quality of adhesion of the dressing.

Study data were collected on body weight, area of resection, operative time, estimated blood loss, hemostatic scores (both before and after application of the chitosan dressing), number of attempts required to securely apply the bandage, and the quality of adhesion.

Statistical Analyses

All data are expressed as mean ± standard deviation (SD) with 95% confidence intervals (95% CI). Comparisons were made between polar resection and wedge resection. The results of parametric data, such as estimated blood loss, operative time, body weight, and area of resection from comparison between polar and wedge resections were analyzed by using the 2-tailed Student *t* test. Mann-Whitney *U* test was used to analyze differences in unpaired nonparametric data, such as number of applications and hemostatic scores. The Wilcoxon signed-rank test was used to compare the hemostatic score between pre- and postchitosan deployment. Chi-square test was used for differences among proportions of urinary leakage in polar and wedge resections. $P \leq 0.05$ was considered statistically significant.

RESULTS

Eighteen partial nephrectomies were performed laparoscopically in 9 animals. A polar resection was performed in 13 kidneys (5 upper poles and 8 lower poles), and 5

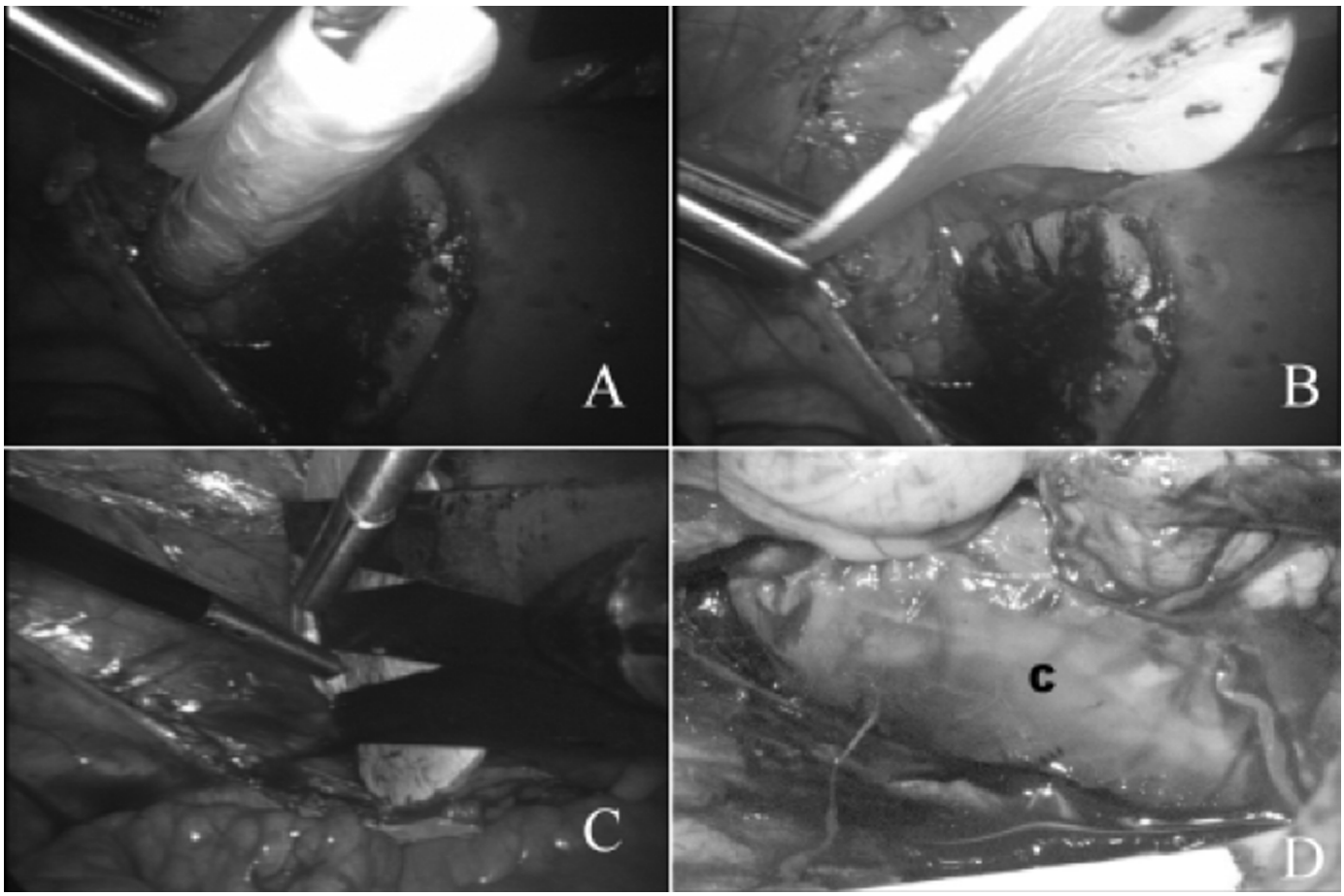

Figure 1. Laparoscopic images present application steps of the chitosan-based hemostatic dressing during LPN procedures: (A) the chitosan dressing was furled and delivered through a 10-mm port; (B) the chitosan dressing was unfurled and deployed onto the renal resected surface with (C) gentle compression using a 10-mm fan retractor for 3 minutes; (D) the gross image shows that the chitosan dressing sealed the renal resected surface (C).

kidneys underwent a wedge resection. The mean blood loss from time of injury until the end of the experimental procedure was 71±104 mL. In 2 animals undergoing wedge resection, the blood loss was in excess of 400 mL. The time and number of attempts required for successful and secure deployment of the dressing following wedge resection were relatively greater than those required for the polar resection; however, the differences were not found to be statistically significant **(Table 1)**.

Following partial resection, steady, continuous bleeding was noted in all instances (hemostasis score 0.8± 0.6). The hemostatic scores improved significantly following successful deployment of the chitosan dressing in 17/18 (94%) procedures (hemostatic score 3.2±1.0 vs. 0.8± 0.6, $P<0.01$). Hemostasis could not be achieved in one case of wedge resection due to the difficulty of deployment of the chitosan dressing. Retrograde pyelography demonstrated no leakage in 14/18 (77.7%) procedures. The dressing failed to prevent urinary extravasation following 2 polar resections (2/13, 15%) and 2 wedge resections (2/5, 40%).

Gross and microscopic examination showed that the chitosan dressings were well adhesed to the renal reseated surface. Histological images showed that the renal resected surface was sealed with the chitosan matrix that gave substantial support for hemostasis and closure of the open collecting system **(Figure 2)**.

DISCUSSION

Nephron-sparing surgery (NSS) has become a standard treatment in patients with solitary kidney or a compromised contralateral kidney, or in select patients with fa-

Table 1.
Operation Data of Laparoscopic Partial Nephrectomy With Chitosan Dressings

	Polar Resection (N = 13)	Wedge Resection (N = 5)	P Value
Resection Weight, g	25 ± 4	19 ± 6	0.19
Number of Applications (range)	1.5 ± 0.7 (1 to 3)	2.4 ± 1.1 (1 to 4)	0.09
Application Time*, min	14 ± 9	27 ± 25	0.29
Estimated Blood Loss, mL, (range)	51 ± 74 (10 to 250)	121 ± 160 (10 to 400)	0.40
Changes of Hemostasis Score (range)	3.2 ± 0.8 (2 to 4)	3 ± 1.7 (0 to 4)	0.50
Urine Leakage (%, n)	(15%, 2/13)	(40%, 2/5)	0.32

*Application time represents the time required for successful deployment of the dressing and achieving secure adhesion of the dressing to the renal parenchymal injury.

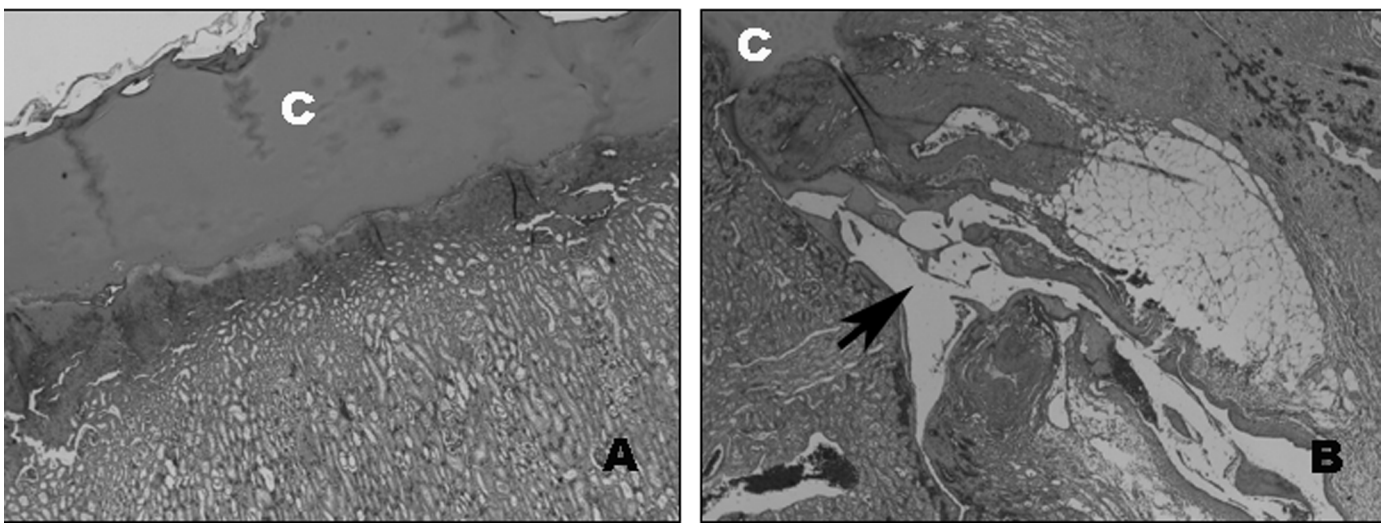

Figure 2. Histological images show that a chitosan-based hemostatic dressing (C) sealed the resection surface of renal parenchyma (A) and the opening collecting system (B, black arrow).

vorably located, small renal tumors with a normal contralateral kidney.[9] Although minimally invasive surgery is becoming established for radical nephrectomy, a similar approach for NSS has been limited largely due to technical issues, such as achieving adequate renal parenchymal hemostasis, and secure caliceal closure.[5] Previous studies showed that overall renal/urological complications and urinary leak-related complications are 25% and 8%, respectively in LPN.[9] Multiple methodologies including suturing closure, thermal coagulation, and topical preparations have been reported to minimize bleeding and urine extravasation in LPN. Suture application is inadequate for diffuse bleeding, and it is difficult to acquire a watertight collecting system seal. Thermal coagulation devices, such as the Harmonic scalpel and argon beam coagulation, are useful tools during renal resection but sometimes hemostasis is incomplete,[8] and it is not possible to close the collecting system. Topical preparations including superficial sealants and hemostatic agents are mostly ineffective while applied to bleeding parenchyma surface and open renal collecting system.[2]

In this study, we looked at the key concerns in capabilities of renal parenchymal hemostasis and watertight sealing of the new chitosan-based hemostatic dressing. Our study demonstrated that the chitosan-based hemostatic dressing is effective in controlling renal parenchymal hemorrhage and pyelocaliceal urinary leakage following LPN. Due to the physical properties of the chitosan dressing, secure application of this material is

feasible and technically less challenging following LPN. For the best results, it is necessary to hold the dressing firmly and steadily against the wound surface of the renal resection for approximately 3 minutes during the operation. In the case of polar resection, we were able to achieve this goal easily with a commercially available fan-type retractor. All of the polar resections achieved complete hemostasis with this technique (100%, 13/13) within a reasonably short duration of time (mean 14 min). Closure of the pyelocalyceal system traditionally requires watertight suture closure. Laparoscopically, this task is technically challenging and time consuming. The ability of the chitosan to adhere strongly to the freshly incised surface provides a unique opportunity to seal the pyelocalceal system. In our study, the chitosan dressing was able to seal the pyelocalceal system securely without any evidence of urinary leakage on follow-up retrograde pyelography in the majority (85%, 11/13) of the polar resections (**Figure 3**). Current clinical data show that the range of mean OR time, blood loss, and complication rate related to bleeding and urine leakage are 179 to 218 minutes, 106 mL to 725 mL, 3.2% to 14% and 1.6% to 13%, respectively **(Table 2)**.[10–15] Our result in these issues was similar to the utilization of traditional hemostatic techniques in LPN according to the literature.[16]

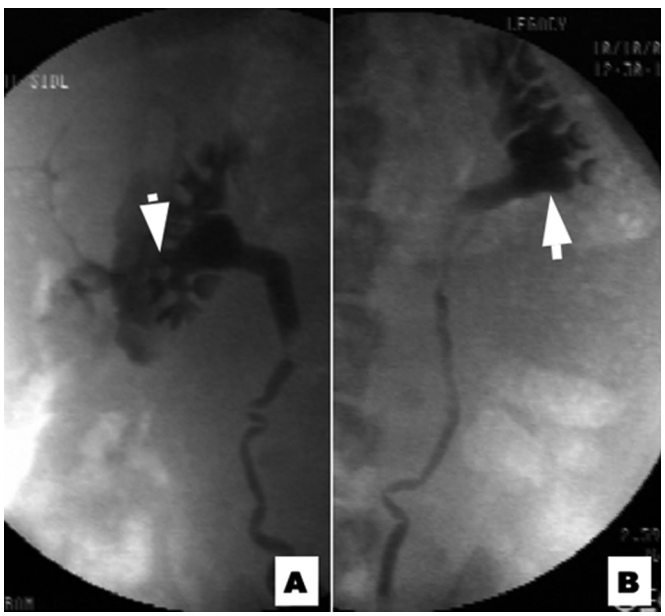

Figure 3. Retrograde ureteropyelogram documenting urinary collecting system after the chitosan dressing deployed. Left image (A) shows an unsuccessful seal with the chitosan dressing in a wedge partial nephrectomy, where gross extravasation of contrast is from the opening of renal calices after the deployment (white arrow). Right image (B) shows that the resection of the pelvis of the kidney has been sealed with the chitosan dressing in a lower polar heminephrectomy (white arrow).

In case of wedge resections, the amount of blood loss and the number of attempts required to apply the dressing were relatively greater. Also the quality of adhesion of the chitosan dressing to the freshly incised renal parenchyma was poorer leading to lower hemostatic scores and a higher rate of urinary leakage (2/5, 40%). In our experience, application of the dressing via the

Table 2.
Summary of Laparoscopic Partial Nephrectomy in Select Literature

Authors	No. Pts	Mean Lesion Size (cm)	Mean OR Time (min)	Mean EBL (mL)	Convert to Open (n)	Complications (n, %)		Hemostatic Technique
						Leak	Bleed	
Rassweiler et al, 2000[10]	53	2.4	191	725	4	5, 9.4	5, 9.4	Bipolar, fibrin glue coated cellulose, Gelatin resorcinol formaldehyde glue
Kim et al, 2003[11]	79	2.5	182	391	1	2, 2.5	5, 6.3	Electrocautery, Polyglactin bolster, suture
Johnston et al, 2005[12]	100	2.5	191	358	3	13, 13.0	9, 9.0	FB, suture, bipolar, ABC, FloSeal®, Gelfoam® bolster
Ramani et al, 2005[13]	200	2.9	199	247	2	4, 2.0	28, 14.0	Monopolar, Surgicel® bolster, suture
Gill et al, 2005[14]	63	2.5	218	106	-	1, 1.6	2, 3.2	Monopolar, Surgicel® bolster, FloSeal®, suture
Weld at al, 2006[15]	60	2.4	179	226	-	5, 8.3	2, 3.3	FB, Harmonic, ABC, Bipolar, Surgicel® Bolster, FloSeal® and suture

EBL = estimated blood loss; FB = fibrin glue; ABC = argon beam coagulation; OR = operating room.

laparoscopic approach was technically challenging following wedge resections due to the awkward "V" shape configuration of the renal parenchymal injury and a lack of appropriate instruments that would allow maintenance of uniform firm pressure over the dressing for 3 minutes. Further developments are required to improve instrumentation for the deployment of the chitosan dressing in laparoscopic wedge resections.

In this study, we used a Harmonic scalpel to dissect the kidney in the heparinized pigs to minimize the bleeding when renal hilar vascular control was not applied. Current literature[8] suggests that even with the use of various energy devices, more than half of the cases fail to acquire satisfactory hemostasis, and suture closure is required to close the pyelocalceal system. In our experience, the use of a Harmonic scalpel did reduce the severity of hemorrhage, allowing us to carry out the entire resection without clamping the renal hilar vasculature. However, the hemorrhage from the freshly incised parenchyma in our anticoagulated porcine model was still serious enough to warrant the need for further hemostatic measures (mean hemostatic score 0.4 and 1 in polar and wedge resection respectively). The hemostatic scores were significantly improved after application of the chitosan dressing in both laparoscopic polar and wedge resection groups. Additionally, the thermal damaged tissue did not adversely affect the adhesion properties of the chitosan dressing.

There are several limitations to this experimental study as a model for partial nephrectomy. Compared with humans, pigs tend to coagulate faster based on their high level of factor V, VII, IX, XI, and XII activities.[17] Although heparin was used to delay clotting time in the pigs, the evaluation of late hemorrhaging may be interfered with by gradual reversion of heparinization. Another main limitation of this study was its design as a nonsurvival experiment. As such, late hemorrhage and urine extravasation were not studied. Future work needs to show the efficacy and biocompatibility of the chitosan dressing in a long-term evaluation.

Comparisons of the chitosan dressing with current available topical hemostatic methods in LPN have not been reported. However, several advantages using this technique seem apparent. The chitosan dressing provides an easy and rapid method to control bleeding and seal the parenchymal wound surface. Use of the chitosan dressing can simplify the LPN procedure to save operative time, and it can be used without hilar vascular occlusion to avoid renal warm ischemia. Clinical situations for possible use of the chitosan dressing in laparoscopic surgery are numerous. Bleeding from visceral organs after routine biopsy and resection, portal bleeding, or more severe bleeding from hilar vascular injury, and surgical bleeding from coagulopathy patients can be controlled using the chitosan dressing as well.

CONCLUSION

This study presents promising initial results for achieving immediate hemostasis and sealing urinary leakage with the use of novel chitosan-based dressing following laparoscopic polar or wedge resection of the kidney in a porcine model. The technique is technically less demanding and allows rapid control of hemorrhage and sealing of the severed pyelocaliceal system. The technique also has several potential applications including laparoscopic control of hemorrhage from solid organs as a result of surgical injury or following trauma. A long-term study for evaluating this new technique is underway.

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